Determination of the partition coefficient (n-octanol/water), HPLC method, of T-450b

Final report

Study no 1612f-19

Contents

Confidentiality statement

Study director's statement

I hereby state, that this study was conducted in accordance with the OECD principles of Good Laboratory Practice (GLP) as administered by the UK Dept of Health and that the report fully and accurately reflects the raw data generated during the study.

All raw data and a copy of the final report will be archived within the Opus Plus facility, on Flotta, for a period of three and a half years from the date of issue of the final report.

Mark Forrest
(Signed)

23 Nov 11
(Date)

Mark Forrest
Study Director
Opus Plus Limited

Quality assurance statement

The conduct of this study has been subjected to inspections by Opus Plus Ltd Quality Assurance Unit. Short term studies are not inspected individually but are subject to process based inspections. The dates of inspection are given below.

Date of QA Inspection	Type of Inspection	Date of Report to Management
17 October 2011	Study plan audit	N/A
31 October – 02 November 2011	Facility inspection	07 November 2011
21 September 2011	HPLC process inspection	23 September 2011
23 November 2011	Report audit	23 November 2011

This report has been audited by the Quality Assurance Personnel according to the appropriate Standard Operating Procedure. The report is considered to describe accurately the methods and procedures used in the study and the original data generated during the study.


(Signed)

23/November/2011
(Date)

Summary

Study details

Sponsor name	Tracerco Limited	**Test personnel**	Mr Mark Forrest, Study Director Sam Archibald, Technician
Sponsor address	Pavilion 11 Belasis Hall Technology Park Billingham Cleveland TS23 4EA	**Test facility**	Opus Plus Limited Flotta, STROMNESS Orkney, KW16 3NP **t** +44 1856 702 000 **f** +44 1856 701 473 admin@opus-results.com www.opus-results.com
Sponsor contact	Rachel Williamson	**Test guidelines**	OECD guideline 117, Partition Coefficient (n-octanol/water), High Performance Liquid Chromatography (HPLC) Method (2004)

Results

Study number 1612f-19 was commissioned by Tracerco Limited to estimate the partition coefficient (n-octanol/water) of T-450b. Due to the material's insoluble properties, it was only possible to obtain an experimental result from the soluble components of T-450b.

The results obtained from tests carried out on 3rd November 2011 are summarised in the table below:

Test material	Number of peaks	log P_{ow} range (first to last peak)	CHARM weighted average log P_{ow}
Soluble components of T-450b	4	1.19 to 6.90	6.7

1 Introduction

The partition coefficient (P) is defined as the ratio of equilibrium concentrations of a dissolved substance in a two-phase system consisting of two largely immiscible solvents. In case of n-octanol and water, the partition coefficient is described according to the following equation:

$$P_{ow} = \frac{\text{test substance concentration (n-octanol)}}{\text{test substance concentration (water)}}$$

P_{ow} is an important parameter in studies of the environmental fate of chemical substances. A significant relationship between the partition coefficient of substances and their bioaccumulation in fish has been shown. In addition, the partition coefficient is a useful tool in the prediction of adsorption on soil and sediments and for establishing quantitative structure/activity relationships for a wide range of biological effects.

This study was performed following the OECD Guideline 117 for testing of chemicals - Partition Coefficient (n-octanol/water), High Performance Liquid Chromatography (HPLC) Method (2004). The method depends upon the principle that when a compound is injected onto a reverse-phase HPLC column its retention time will be governed by its hydrocarbon-water partition coefficient. Log P_{ow} values for test materials can therefore be calculated utilising a calibration curve prepared from a selected range of reference compounds with known log P_{ow} values.

In this study, the partition coefficient is deduced from the capacity factor k:

$$k = \frac{t_R - t_0}{t_0}$$

where, t_R is the retention time of the test substance and t_0 is the dead-time, ie the average time the un-retained reference component needs to pass the HPLC column.

The measured capacity factor k of the test substance is correlated to its log P_{ow} using the calibration curve obtained for the selected reference substances. Reference materials were selected in the log P_{ow} range of 0.3-6.5 log P_{ow} values outside this range are estimated by extrapolation.

When carrying out HPLC analysis (OECD Guideline 117) it is very important to ascertain whether the chemical is suitable or not. Where a material is found to be insoluble or does not produce any detectable presence, they are classed as unsuitable for this method of testing.
This leads on to the possibility of calculating an estimated theoretical log P_{ow} value which can be achieved by using the chemical structural formula of a specific test material or CAS number as the input to a model.

2 Reference materials and equipment

2.1 Preparation of reference standards

The following reference compounds were obtained from commercial suppliers and stored according to suppliers instructions:

		literature Log P_{ow} values
• Thiourea	CAS [62-56-6]	
• 2-Butanone	CAS [78.93.3]	0.3
• Benzene	CAS [71-43-2]	2.1
• Toluene	CAS [108-88-3]	2.7
• Naphthalene	CAS [91-20-3]	3.6
• Fluoranthene	CAS [206-44-0]	5.1
• 4,4'-DDT	CAS [50-29-3]	6.5

2.1.1 Log P_{ow} reference standard

A reference solution was prepared by dissolving the reference materials in 75/25 (v/v) methanol/water eluent at the following approximate concentrations. This solution was injected onto the HPLC column and the average retention times for the reference materials were calculated from duplicate runs.

- Benzene 301 mg/l
- Toluene 302 mg/l
- Naphthalene 300 mg/l
- Fluoranthene 302 mg/l
- 4,4'-DDT 302 mg/l

A separate solution of the reference material 2-butanone was prepared in eluent in combination with thiourea (see below) and run in duplicate on the HPLC. The average retention time was calculated from duplicate runs.

- 2-Butanone 4755 mg/l

2.1.2 Dead time reference standard

Dead time (t_o) was measured using a solution of thiourea (1045 mg/l) which was freshly prepared in eluent on the day of testing. The average retention time for the thiourea was calculated from duplicate runs.

2.2 Equipment and reagents

In this study, the following conditions were used:

Eluent:	75/25 (v/v) methanol/distilled water
Injection:	100 µl sample loop
Column:	Microsorb-MV 100 C18, 5 µm (250 mm x 4.6 mm)
Pump:	Shimadzu LC-6A isocratic pump
Detector:	Bischoff 8120 differential refractive index (RI)
Flow rate:	1.0 ml/minute
Temperature:	Room temperature, varying no more than ±2 ºC during each day of testing

3 Calibration and calculations

In order to correlate the measured capacity factor, k with the P_{ow} value a calibration graph was prepared by plotting measured log k values versus the known literature values of log P_{ow} for the reference substances. The log P_{ow} of peaks recorded within the range of retention times of the reference substances were calculated by interpolation of the calculated capacity factor k on the calibration curve. Estimates of log P_{ow} for peaks eluting outside the reference standard range were obtained by extrapolation of this calibration curve.

For many test substances, HPLC analysis results in detection of more than one peak in the chromatogram. In these cases the log P_{ow} for the test material is calculated as follows:

The area percent and the log P_{ow} values were calculated for all peaks greater than 1% of the total area detected. A weighted average log P_{ow} calculation was then carried out according to the CHARM method:

CHARM weighted average log P_{ow} = log Σ (P_{ow}(peak i) x area % (peak i)/100).

The reported weighted average log P_{ow} for the test material is calculated from the average retention time and peak area data obtained from duplicate runs. In the case of materials containing more than one component, the log P_{ow} range was determined using the average retention times of the first and the last peaks detected.

4 Test material: T-450b

4.1 Test material description and preparation for analysis

The test material T-450b was received from the client by Opus Plus Ltd, Flotta and stored at room temperature in the dark. The material was characterised and prepared for analysis as shown in Tables 4.1 and 4.2.

Table 4.1 Description and characterisation (SOP 402)

Property	MSDS supplied	Observed
Form	Liquid	Liquid
Colour	Colourless	Clear/Colourless
Density	No data available	1.8656g/cm^3
Odour	Odourless	None
Viscosity	2mPa.s (approx.)	Slight
pH	Not applicable	DiW = 5.52 @1000mg.l^{-1}
Solubility	Not miscible	Insoluble
Flash point	No data available	
Melting point	No data available	
Boiling point	130°C	
Chemical descriptions	Name, CAS number, Percentage composition	
	Perfluoro-n-propylcyclohexane	
	CAS No 420-02-9	
	Percentage composition: >99%	

Table 4.2 Test solution preparation

Test solution preparation	
Concentration (g.l^{-1})	Solvent
74.2	Methanol

The test material appeared poorly soluble in both eluent and methanol after five minutes in an ultra sonic bath. Therefore a sample was taken from the mid-column and the methanol test solution was injected into the HPLC system. The test was repeated because it required to be run longer than the usual 70 minutes and also required negative polarity. The results for the soluble components of T450b are stated below.

4.2 Results

The calibration graph and reference standard data used for this determination is displayed in Figure 4.1 with chromatographic profiles shown in Figure 4.2. The chromatograms for the test material in Figure 4.2 show four peaks. Negative polarity was used for the test material runs. Calculations were performed on duplicate analytical results obtained for the test material and are given in Table 4.3.

The mean CHARM weighted average log P_{ow} for the soluble components of T-450b was calculated to be 6.7.

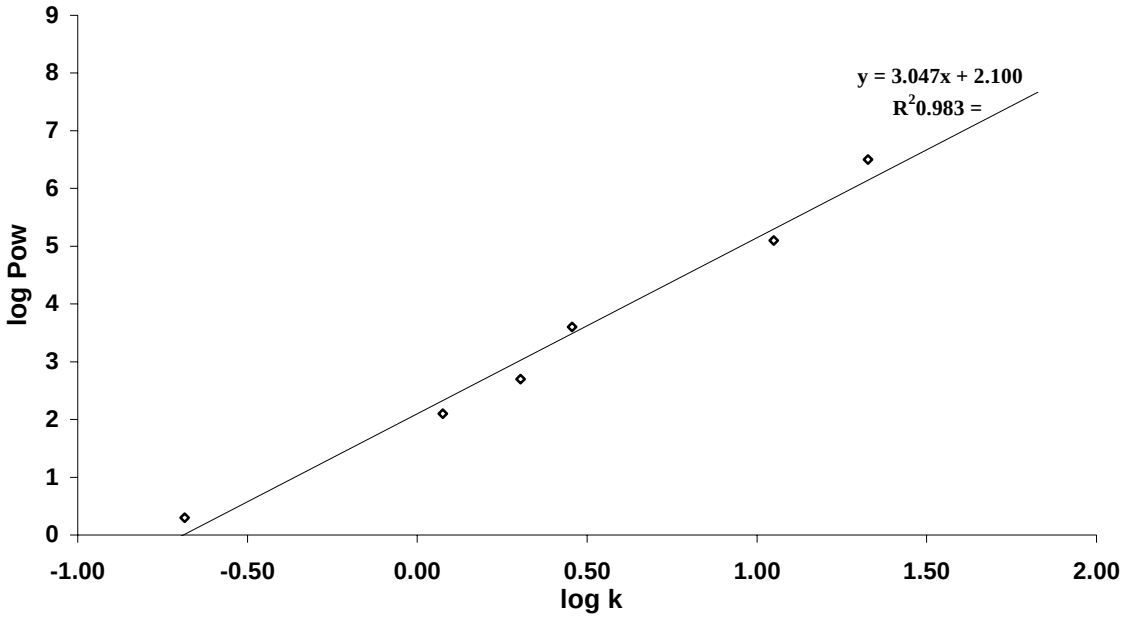
The range of log P_{ow} for the four peaks was from 1.19 to 6.90.

A theoretical log P_{ow} value could not be calculated as the CAS number 420-02-9 was not recognised by Episuite.

Figure 4.1 Calibration graph

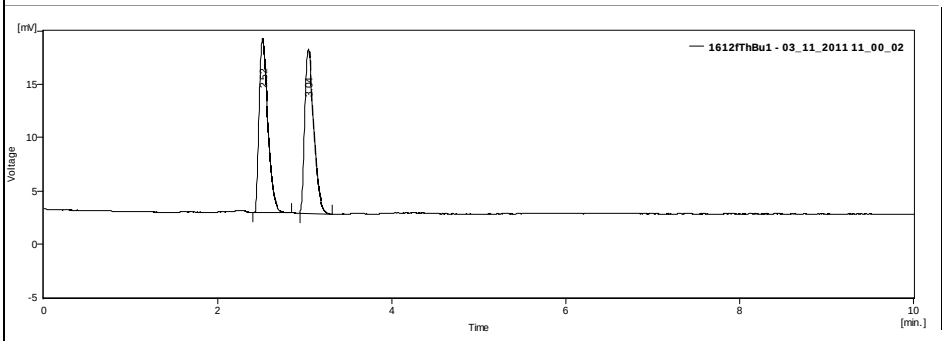
calibration date *03 November 2011*

Calibration data

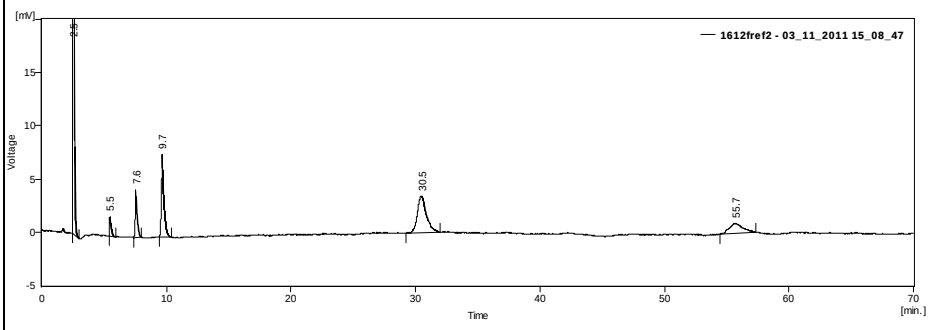


Reference Material	tR (min)	log k	Theoretical
Thiourea	2.52		
2-Butanone	3.04	-0.69	0.3
Benzene	5.52	0.07	2.1
Toluene	7.60	0.30	2.7
Naphthalene	9.72	0.46	3.6
Fluoranthene	30.73	1.05	5.1
4,4'-DDT	56.10	1.33	6.5

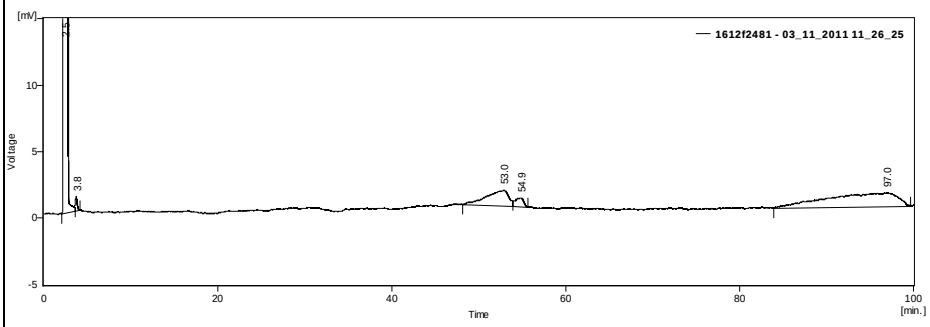
Figure 4.2 Chromatograms: T-450b



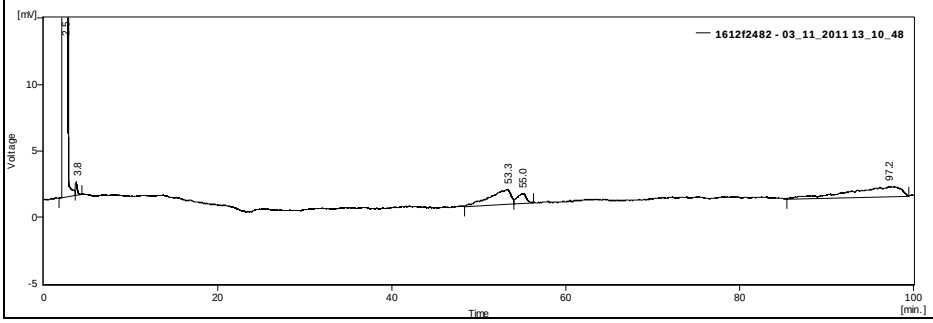
Thiourea (dead time) + 2-Butanone (Ref std)	
Peak	*tR (min)*
Thiourea	2.52
2-Butanone	3.04



Reference standard	
Peak	*tR (min)*
Benzene	5.52
Toluene	7.60
Naphthalene	9.72
Fluoranthene	30.73
4,4'-DDT	56.10



Sample injection 1	
Peak	*tR (min)*
1	3.79
2	52.95
3	54.91
4	96.97



Sample injection 2	
Peak	*tR (min)*
1	3.78
2	53.32
3	55.02
4	97.17

Table 4.3 Calculations: T-450b

Injection 1 *Filename 1612f2481*

Date	Peak No	tR (min)	%Area	k	log k	log Pow	Pow	weighted Pow
3 Nov 11	1	3.79	2	0.50	-0.30	1.19	15.6	0.3
	2	52.95	25	20.01	1.30	6.07	1161517.7	290379.4
	3	54.91	5	20.79	1.32	6.12	1304615.2	65230.8
	4	96.97	68	37.48	1.57	6.90	7859102.1	5344189.4

Injection 2 *Filename 1612f2482*

Date	Peak No	tR (min)	%Area	k	log k	log Pow	Pow	weighted Pow
3 Nov 11	1	3.78	3	0.50	-0.30	1.18	15.2	0.5
	2	53.32	32	20.16	1.30	6.07	1187679.6	380057.5
	3	55.02	10	20.83	1.32	6.12	1312979.5	131298.0
	4	97.17	55	37.56	1.57	6.90	7909919.7	4350455.9

Average values

Date	Peak No	tR (min)	%Area	k	log k	log Pow	Pow	weighted Pow
3 Nov 11	1	3.79	3	0.50	-0.30	1.19	15.42	0.4
	2	53.14	29	20.09	1.30	6.07	1174549.71	334746.7
	3	54.97	8	20.81	1.32	6.12	1308792.85	98159.5
	4	97.07	62	37.52	1.57	6.90	7884483.41	4848957.3

MEAN CHARM weighted average Log Pow 6.7

Appendix

References

OECD Guidelines for Testing of Chemicals Section 1, Guideline 117. Partition Coefficient (n-octanol/water), High Performance Liquid Chromatography (HPLC) Method (adopted 13 April, 2004).

CHARM Technical Note 61. November 1996. Calculation of Log Weighted Average Log P_{ow}.